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REVIEW

The Origin, Differentiation, and Functions of Cancer-Associated Fibroblasts in Gastrointestinal Cancer

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SUMMARY

We summarize the newest understanding of gastrointestinal cancer-associated fibroblasts with a special focus on their origin, differentiation, and function. We also discuss the most advanced knowledge on cancer-associated fibroblast subsets as revealed by single-cell technologies.

Emerging evidence has shown the importance of the tumor microenvironment in tumorigenesis and progression. Cancer-associated fibroblasts (CAFs) are one of the most infiltrated stroma cells of the tumor microenvironment in gastrointestinal tumors. CAFs play crucial roles in tumor development and therapeutic response by biologically secreting soluble factors or structurally remodeling the extracellular matrix. Conceivably, CAFs may become excellent targets for tumor prevention and treatment. However, the limited knowledge of the heterogeneity of CAFs represents a huge challenge for clinically targeting CAFs. In this review, we summarize the newest understanding of gastrointestinal CAFs, with a special focus on their origin, differentiation, and function. We also discuss the current understanding of CAF subpopulations as shown by single-cell technologies. ***(Cell Mol Gastroenterol Hepatol 2023;16:503–511; https://doi.org/10.1016/j.jcmgh.2023.07.001)***

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Gastrointestinal cancer is one of the diseases that seriously endanger human health. Recent studies have found that cancer is not only a disease caused by genetic or epigenetic changes in tumor cells, but also a systemic disease caused by the body's response to malignancy. Although cancer cells themselves play an important role in tumor development and progression, other cells in the tumor microenvironment (TME), such as endothelial cells, immune cells, and fibroblasts, also contribute significantly to the process of tumorigenesis and chemoradiotherapy response.[1] In pancreatic ductal adenocarcinoma (PDAC), up to 90% of the tumor consists of extracellular matrix (ECM) and stromal cells including fibroblasts.[2] Therefore, it is critical to show the biological role of TME in tumor development. Fibroblasts are the predominant cell type within the cancer stroma and are known as cancer-associated fibroblasts (CAFs). Numerous studies have reported that CAFs are histologically prominent and functionally important in the initiation and progression of gastrointestinal cancer.[3–5] For instance, CAFs are one of the most abundant cell types in TME, and they can build and remodel the structure of extracellular matrix.[6] CAFs also participate in the co-evolution of tumor cells and other stromal cells by producing multiple cytokines.[7] In addition, studies have found that CAFs are major contributors to tumor invasion and metastasis.[3,8] Therefore, the abundance of CAFs often significantly affects patient prognosis. A retrospective study has shown that in patients with colorectal cancer after surgery, the content of CAFs in tumor tissue is correlated negatively with disease-free survival ($P = .001$), which can be used to predict the recurrence of colorectal cancer.[9] Consistently, single-cell analysis has found a subtype of CAFs with high expression of Inhibin beta-A (INHBA) and Fibroblast activation protein alpha (FAP) as a predictor of poorer survival in gastric cancer (GC).[10] With the deepening of the understanding of CAFs, the role of CAFs in tumorigenesis and their potential therapeutic value have attracted more and more attention. Conceivably, CAFs may become excellent targets for tumor prevention and treatment. The clinical implementation of CAFs in gastrointestinal cancer is well-described elsewhere.[5] In this review, we outline the latest progress in the origin, differentiation, and functions of CAFs.

Abbreviations used in this paper: **apCAF, antigen-presenting cancer-associated fibroblast; α-SMA, alpha-smooth muscle actin; CAF, cancer-associated fibroblast; COL1A1, collagen type I alpha 1 chain; CRC, colorectal cancer; CXCL, C-X-C motif chemokine ligand; ECM, extracellular matrix; EGF, epidermal growth factor; ESCC, esophageal squamous cell carcinoma; FAP, fibroblast activation protein alpha; FSP1, fibroblast-specific protein 1; GC, gastric cancer; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; HSC, hepatic stellate cell; iCAF, inflammatory cancer-associated fibroblast; ICC, intrahepatic cholangiocarcinoma; IGF, insulin-like growth factor; IL, interleukin; INHBA, inhibin subunit beta a; JAK, Janus kinase; MSC, mesenchymal stem cell; Msi, musashi rna binding protein; myCAF, myofibroblastic cancer-associated fibroblast; PDAC, pancreatic ductal adenocarcinoma; PRKCZ, protein kinase c zeta; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor β; TME, tumor microenvironment.**

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Origin of CAFs

Because of the lack of CAFs-specific markers, the origin of CAFs remains controversial. Several studies have indicated that bone marrow–derived mesenchymal stem cells (MSCs) can be converted into CAFs.[11,12] Transplantation of MSCs with Alpha-smooth muscle actin (*α-SMA*) reporter show that approximately 20% of CAFs originate from bone marrow–derived MSCs in a mouse model of gastric carcinoma.[12] This has been confirmed in patients who developed gastric and rectal cancer after bone marrow transplantation.[11] However, other experimental studies have shown that CAFs derive predominantly from the activation of local tissue-resident fibroblasts.[13] By using 3 independent approaches including skin graft models, parabiosis, and bone marrow transplantation, Arina *et al*.[13] found that α-*SMA*$^{+}$ and collagen type I α1 chain (*COL1A1*)$^{+}$ CAFs derive primarily from local fibroblast precursors rather than circulating cells. Evidence for other sources of CAFs has been reported in different cancer types and animal models, although they are not universally applicable. Exogenous hepatic progenitor cells have been shown to differentiate into myofibroblasts in hepatocarcinogenesis.[14] It also has been suggested that tumor stromal cells originate from adipocytes[15,16] and epithelial cells,[17,18] but there is no direct evidence to support their transdifferentiation to CAFs.

Genetic lineage tracing in mice is a useful and convincing tool for exploring the source of CAFs. Because of the limited knowledge about specific gene markers of fibroblasts, it remains difficult to select a promoter to drive the expression of Cre recombinase. It is worth noting that this situation has been improved to some extent by the advent of single-cell sequencing technology, which can specifically find markers that distinguish cell clusters. Hepatic stellate cells (HSCs) have been suggested to be the source of CAFs in hepatocellular carcinoma (HCC), however, formal fate-tracing studies remain hampered because of the lack of highly specific markers for normal HSCs. In a recent study, Transcription factor 21 (*Tcf21*) was identified as an HSC-specific gene by single-cell sequencing on enriched nonparenchymal cells from enzymatically dissociated mouse livers. Then, fate-mapping of *Tcf21*$^{+}$ cells showed that approximately 85% of CAFs derive from *Tcf21*$^{+}$ HSCs.[19] In addition, by applying a lineage-tracing strategy in various mouse lines, it was shown that 53% of proliferating Actin alpha 2, smooth muscle (*ACTA2*)$^{+}$ CAFs stem from intestinal pericryptal leptin-receptor–positive cells during Azoxymethane (AOM)/Dextran Sodium Sulfate (DSS) colorectal tumorigenesis.[20]

Currently, it remains unclear whether the origination of CAFs is preserved across various tissues and species. The consensus is that the predominant source of CAFs is native precursors, but a small proportion of CAFs are derived from other ancestors. The different functions of CAFs from different sources remain to be elucidated.

Differentiation

Several signaling molecules have been shown to promote the generation of CAFs. Transforming growth factor β (*TGFβ*) is well characterized in the process of CAF differentiation.[21,22] Autocrine-*TGFβ* is involved in the initiation and differentiation of fibroblasts into myofibroblasts and the concurrent tumor-promoting phenotype.[21] CAF differentiation is influenced mainly by tumor-derived signals. Direct contact between tumor cells and fibroblasts could contribute to CAF differentiation via Notch signaling.[23]

Soluble modulators such as osteopontin also play an important role in CAF reprogramming.[24] Intercellular crosstalk between fibroblasts and tumor cells involving nuclear factor-κB signaling and interleukin (*IL*)*6*/*IL8* further promotes CAF activation.[25] Cues from other stromal components also may educate fibroblasts. For example, macrophage-derived granulin activates resident hepatic stellate cells, resulting in a liver fibrotic microenvironment that supports metastatic tumor growth of PDAC.[26]

Intrinsic factors mediating fibroblast-to-myofibroblast transdifferentiation have been studied in detail. Protein kinase C zeta (*PRKCZ*) is critically and negatively involved in this process.[27] Loss of *PRKCZ* in colonic fibroblasts is instrumental for the induction of SRY-box transcription factor 2 (*SOX2*), which is an essential contributor to the CAF phenotype in colorectal cancer (CRC). In addition, P300 acetyltransferase is critical for *TGFβ1*-mediated HSC differentiation into metastasis-promoting myofibroblasts.[28] Moreover, NADPH oxidase 4 (*NOX4*) has been implicated in the CAF differentiation in colorectal cancer.[29] Conceivably, the process of CAF differentiation is a potentially attractive therapeutic target in the future.

Functions of CAFs

Co-injection of fibroblasts and tumor cells is a traditional and simple way to study the function of CAFs. However, this method is only suitable when the experiment duration is short enough because host-derived fibroblasts would grow over the injected fibroblasts. A transgenic mouse model is another way to study the function of fibroblasts in vivo. Different from the co-injection method, using Cre/lox-site-specific recombination (Cre–lox) systems to modulate CAFs helps monitor the long-term effects of CAFs without disturbance of fibroblasts with mixed origins. For example, using Col1a1 as a marker of myofibroblasts in the liver, transgenic mice (Musashi RNA binding protein 2, *Msi2*$^{\Delta Col1a1}$) with floxed *Msi2* allele and *Col1a1*-ligand–inducible Cre recombinases have been constructed to generate a CAF-specific *Msi2* knockout model,[30] which showed that myofibroblast-specific *Msi2* knockout abrogated the tumor-promoting function of myofibroblasts in vivo. Fibroblast-specific protein 1 (*FSP1*; also called *S100A4*) is another commonly used marker for fibroblast. By crossing *Fsp1*–Cre mice with *Prkcz*$^{f/f}$ mice to achieve deletion of the *Prkcz* gene in fibroblasts, a study about CRC showed that loss of *Prkcz* in fibroblasts promoted tumorigenesis.[27] In addition, Collagen VI (*ColVI*)–Cre mice were crossed with signal transducer and activator of transcription (*Stat*)*3*$^{fl/fl}$ mice to achieve *STAT3* inactivation in fibroblasts during CRC carcinogenesis. This uncovers the key role of *STAT3* in CAFs for the growth of colon tumors in vivo.[31] However, none of the markers is expressed specifically by fibroblasts. *FSP1* is expressed by subsets of myeloid cells, *COL1A1* is expressed in osteoblasts, and ColVI

also can be detected in the peripheral nervous system. These advantages and disadvantages should be weighed when deciding which method to use.

CAFs have been implicated to play important roles in promoting tumor growth and aggressiveness,[32–34] interacting with other components in tumor mass,[35–37] impacting chemoradiotherapy response,[38–40] and regulating tumor immunology[41–43] (Figure 1). Of note, CAFs are a highly heterogeneous cell population with various functions in different studies. This may be caused by various combinations of CAF subtypes with different properties. Before the occurrence of single-cell technologies, it was difficult to dissect multiple subsets of CAFs, explore the markers defining them, and uncover multifaceted roles of different CAF subsets. In recent years, single-cell sequencing has largely deepened our knowledge about the biological diversity of CAFs and promoted the development of cancer treatment strategies targeting CAFs. Representative single-cell studies showing CAF subsets and features are listed in Table 1, which shows that different cancer types share some common CAF subsets. In this section, we discuss various CAF functions in multiple gastrointestinal cancers and summarize the most advanced knowledge on CAF subsets as shown by single-cell technologies.

Pancreatic Cancer

The secretome of CAFs greatly impact PDAC. Exosomal microRNA (miR)-10a-5p released by CAFs have supportive effects on the growth of pancreatic cancer cells.[49] Moreover, soluble factors from CAFs including insulin-like growth factor (*IGF*)[50] and nitric oxide[51] mediate PDAC resistance to chemotherapy. PDAC has highly desmoplastic stroma, which is unfavorable for drug penetration. One of the main reasons might be CAF-induced high interstitial fluid pressure and substantial barriers to drug penetration.[39] In addition, CAFs have a great impact on the establishment of an immunosuppressive microenvironment.[52] Mechanistically, CAFs directly act on $CD8^+$ T-cell infiltration through modulation of chemokine levels.[41] Another study in a genetically engineered model of PDAC showed that FAP-expressing CAFs

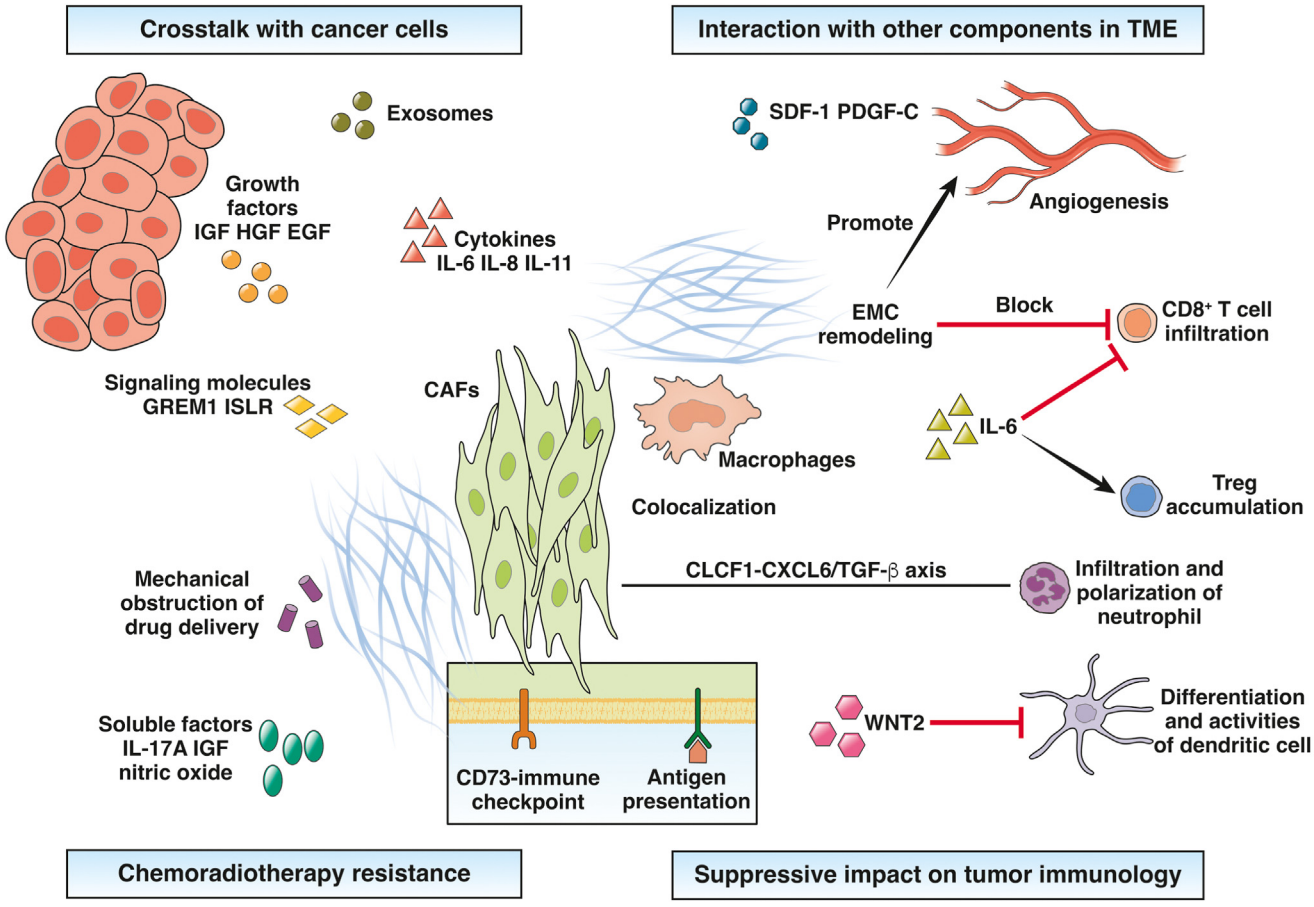

Figure 1. Multifaceted functions of CAFs. CAFs are histologically prominent and functionally important in the initiation and progression of gastrointestinal cancer. They are a major source of cytokines, growth factors, signaling molecules, and exosomes, which mediate crosstalk between cancer cells. In addition, CAFs interact closely with other components in the TME. As a consequence, CAFs promote angiogenesis and suppress tumor immunology. Moreover, CAFs could act as antigen-presenting cells and express immune checkpoints, which further inhibit immunologic response. Finally, CAFs induce therapeutic resistance by active biochemical signaling or mechanical obstruction of drug delivery. CLCF1, cardiotrophin like cytokine factor 1; GREM1, gremlin 1; ISLR, immunoglobulin superfamily containing leucine rich repeat; PDGF-C, platelet-derived growth factor C; SDF-1, stromal cell–derived factor 1; Treg, regulatory T cells.

Table 1. Representative Single-Cell Studies Showing CAF Subsets and Features

Study	Organism	Fibroblasts, n	Subsets	Markers	(Predicted) Functions or pathways
Ela Elyada et al,[44] PDAC	KPC mouse model and human PDACs	962 (human) and 4012 (mice)	Inflammatory CAFs	Clec3b, Ly6c1, Cxcl12, Cxcl1, Ccl7, Il6, Has1, Col14a1	Up-regulation of inflammatory pathways, ECM deposition
			Myofibroblastic CAFs	Acta2, Tagln, Igfbp3, Thy1, Col12a1, Thbs2	EMT, myogenesis, ECM-receptor interaction, focal adhesion
			Antigen-presenting CAFs	H2-Aa, H2-Ab1, Cd74, Saa3, Slpi	Antigen presentation and processing, fatty acid metabolism, MYC targets, MTORC1 signaling
Kai Chen et al,[45] PDAC	Human PDAC with different clinical stages	2958	Classic CAFs	COL1A1, LUM (extracellular matrix–related components), MMP11 (matrix remodeling molecular), FAP (stromal fibroblast activated protein), and SFRP2 (modulators of Wnt signaling)	Extracellular matrix deposition
			Complement-secreting CAFs	C3, C7, CFB, CFD, CFH, CFI	Regulate immune and inflammation response within the tumor
Huipeng Li et al,[46] CRC	Human CRC	26	CAF-B	ACTA2, TAGLN, PDGFA	NA
			CAF-A	MMP2, DCN, COL1A2	NA
Min Zhang et al,[47] ICC	Human ICC	2941	Vascular CAFs	CD146 (MCAM), MYH11, GJA4, RGS5, and inflammatory chemokines (IL6 and CCL8)	Muscle contraction, response to hypoxia, and mesenchymal cell proliferation
			Matrix CAFs	Collagen molecules (COL5A1, COL5A2, and COL6A3), periostin (POSTN), FN1, LUM, DCN, and VCAN	ECM and collagen fibril organization
			Inflammatory CAFs	FBLN1, IGFI, CXCL1, IGFBP6, SLPI, SAA1, and complement genes (C3 and C7)	ECM, inflammatory response regulation, and complement activation
			Antigen-presenting CAFs	MHC-II genes (CD74, HLA-DRA, HLA-DRB1)	Leukocyte cell–cell adhesion, response to IFN-γ, antigen processing, and antigen presentation via MHC-II
			EMT-like CAFs	Epithelium-specific marker genes such as *KRT19*, *KRT8*, and *SAA1*	NA
			Lipofibroblast (mainly derived from adjacent tissues)	Lipid metabolism–related genes, including *APOA2*, *FABP1*, *FABP4*, and *FRZB*	Lipid metabolism and processing
Yongxu Jia et al,[48] ESCC	Human ESCC	7857	Inflammatory CAFs	IL6, HAS1, COL14A1	NA
			Myofibroblastic CAF-1	THBS2, COL12A1, THY1, IGKC	NA
			Myofibroblastic CAF-2	THBS2, COL12A1, THY1, COL1A1, CTHRC1, MMP1, and MMP11	NA
			Antigen-presenting CAFs	CD74, HLA-DRA, and HLA-DPB1	NA

CCL, c-c motif chemokine ligand; CTHRC1, collagen triple helix repeat containing 1; EMT, epithelial-mesenchymal transition; FN1, fibronectin 1; GJA4, gap junction protein alpha 4; HAS1, hyaluronan synthase 1; IFN-γ, interferon-γ; KPC, $Kras^{G12D/+}$; $Trp53^{R172H/+}$; P48-Cre; LUM, lumican; MCAM, melanoma cell adhesion molecule; MHC, major histocompatibility complex; MMP, matrix metalloproteinase; MTORC1, mammalian target of rapamycin complex 1; MYC, MYC proto-oncogene, bHLH transcription factor; MYH11, myosin heavy chain 11; PDGF, platelet-derived growth factor; RGS5, regulator of G-protein signaling 5; SFRP2, secreted frizzled related protein 2; TAGLN, transgelin; THBS2, thrombospondin 2; THY1, Thy-1 cell surface antigen.

are a main source of chemokine (C-X-C motif) ligand (*CXCL*) *12*, which mediate immune suppression via inhibiting T-cell accumulation.[53]

Single-cell analysis from mouse and human PDAC showed the presence of myofibroblastic CAFs (myCAFs), inflammatory CAFs (iCAFs), and antigen-presenting CAFs (apCAFs).[44] myCAFs express a high level of α-*SMA*. These cells showed high expression of smooth muscle genes such as *Acta2* and *Tagln*. By contrast, iCAFs express low levels of α-*SMA*, but high levels of cytokines and chemokines such as *Il6* and *Cxcl1*, as well as ECM deposition–related genes including *Has1* and *Col14a1*. apCAFs is a newly identified subtype that could activate $CD4^+$ T cells in an antigen-specific fashion. These CAFs express genes belonging to the major histocompatibility complex class II family, which usually only are present on antigen-presenting cells, such as dendritic cells. For example, *H2-Aa* and *H2-Ab1* genes were identified as marker genes of apCAFs, which encode the α and β chains of major histocompatibility complex class II of C57BL/6 mice. In vitro culture systems further identified that TGFβ and *IL1*/Janus kinase [*JAK*]/*STAT* signaling are responsible for the induction of myofibroblastic and inflammatory phenotypes in CAFs, respectively. In addition, *TGF*β signaling antagonized *IL1*-induced iCAF formation.[54] Using lineage tracing strategies and pseudotime analysis of single-cell transcriptomes, scientists found that apCAFs are derived from mesothelial cells in pancreatic cancer. Moreover, the subtype of apCAFs could directly induce naive $CD4^+$ T cells to differentiate into regulatory T cells in an antigen-specific manner, which contributes to immune evasion in pancreatic cancer. Importantly, targeting the transition process from mesothelial cells to apCAFs may be promising to enhance cancer immune therapy.[55] Interestingly, another single-cell study found that myCAFs could be detected in precursor lesions of PDAC. Moreover, more myCAFs are present in higher-grade human intraductal papillary mucinous neoplasia (a common precursor lesion of PDAC), which indicates that activation of fibroblasts to the myCAFs phenotype may be involved in the progression from noninvasive intraductal papillary mucinous neoplasia to PDAC.[56] It is worth noting that the composition of CAFs could change in different stages of cancer. A single-cell study on genetically engineered mouse models including normal pancreas, early PDAC, and late PDAC showed that normal and early PDAC pancreas contain 3 fibroblast populations, whereas late PDAC contains only 2.[57]

Beyond the earlier-mentioned 3 types of CAFs, a kind of complement-secreting CAFs also has been identified. These CAFs highly expressed genes involved in the complement system, including *C3*, *C7*, *CFB, CFD, CFH, and CFI*, which may regulate immunologic responses within the tumor.[45] Another study using mass cytometry and single-cell technology showed that *CD105* demarks 2 functionally distinct CAF subpopulations in murine and human PDAC samples.[58] *CD105*-positive fibroblasts are permissive for tumor growth in vivo. However, *CD105*-negative fibroblasts restrict tumor growth in vivo, which is entirely dependent on functional adaptive immunity.[58] This study may explain why genetic depletion of CAFs induced immunosuppression and accelerated the progression of pancreas cancer.[59]

Colorectal Cancer

CAFs are a major source of cytokines that regulate tumor growth and aggressiveness. CAF-produced *IL6/IL8* have been implicated in CRC aggressiveness.[32] As a result, CAFs make CRC resistant to bromodomain and extraterminal inhibitors through the *IL6/IL8–JAK2*–bromodomain containing 4 (*BRD4)* cascade. Expression of *IL6/IL8* also promotes cancer metastasis.[33] Through the secretion of *IL11*, CAFs activate both tumor cells and fibroblasts in the tumor microenvironment. Moreover, genes enriched in $IL11^+$ fibroblasts are correlated with reduced recurrence of disease-free durations of CRC.[60] CAFs also produce gremlin 1 (*GREM1*) and immunoglobulin superfamily containing leucine rich repeat (*ISLR*), which balance stromal bone morphogenetic protein (BMP) signaling and contribute to colorectal carcinogenesis.[34] In intestinal tumors, a population of prostaglandin-endoperoxide synthase 2 (*Ptgs2*)-expressing fibroblasts could constitutively produce prostaglandin E 2 and promote the expansion of tumor-initiating cells via the prostaglandin E 2–prostaglandin E receptor 4 (Ptger4)–Yes-associated protein (Yap) signaling axis.[61]

In addition to being a substantial source of secreted factors, CAFs themselves could act as nonprofessional antigen-presenting cells and suppress immune responses in the tumor microenvironment. Cross-presentation of antigens by CAFs are shown in CRC-derived CAFs, which suppress $CD8^+$ T-cell function in an antigen-dependent manner. However, unlike professional antigen-presenting cells such as dendritic cells, these antigen-presenting CAFs lack expression of co-stimulatory molecules (*CD80/CD86*) and suppress tumor-specific $CD8^+$ T-cell function.[62]

Several studies have provided evidence that exosomes participate in the interaction between tumor cells and CAFs. CAF-derived exosomes promote metastasis and chemotherapy resistance of CRC.[63] In addition, they have prognostic value in colon cancer patients.[64] Apart from tumor cells, CAFs have a lot of physical interactions and biological crosstalk with other constituents in TME. Neovascularization is one of the hallmarks of cancer.[65] Metastasis-associated CAFs induce endothelial cell sprouting and thus support angiogenesis by enhancing matrix stiffness. Therefore, the reduction of CAF-induced stiffness can increase the efficacy of anti-angiogenic therapy.[66] Other cell components also are associated closely with CAFs. Single-cell spatial transcriptomics show near-localization of FAP^+ fibroblasts and (Secreted phosphoprotein 1) $SPP1^+$ macrophages in colorectal cancer. This interaction may facilitate the remodeling of ECM, which potentially limits T-cell infiltration into the tumor nests.[37]

CAFs have been found to play an important role in interfering with therapy response. Chemotherapy-induced enrichment of CAFs promotes cancer cell self-renewal and chemotherapy resistance through the action of *IL17A*.[67] During radiotherapy, CAFs can be activated and further promote tumor progression via paracrine *IGF1R* activation.[40] It is worth noting that CAFs are the main type of cells responsible for ECM remodeling. Abnormal accumulation of extracellular matrix components leads to immunosuppression and immunotherapy resistance. Transcriptional regulator heat shock factor 1 plays a crucial role in orchestrating

ECM structure and composition.[68] *TGFβ*, derived mainly from CAFs, promotes T-cell exclusion and blocks the acquisition of the Type 1 T helper (TH1)-effector phenotype in CRC mouse models. Importantly, the blockade of TGFβ signaling synergizes with anti-programmed cell death protein 1 (*PD1*)/programmed cell death ligand 1 (*PD-L1*) immunotherapy.[69] In addition, *CD73* on CAFs serves as an immune checkpoint in CRC, and *CD73* neutralizing antibody enhances antitumor immunity in CAF-rich tumors.[70]

In a single-cell study of human primary colorectal cancer, there were 2 subsets of CAFs identified (CAF-A and CAF-B). Differential expression analysis showed that CAF-B cells express markers of myCAFs as described in PDAC, including *ACTA2*, transgelin (*TAGLN*), and platelet-derived growth factor A. By contrast, these markers are decreased in CAF-A cells, which express matrix metalloproteinase (*MMP*)*2*, decorin (*DCN*), and *COL1A2*.[46] Recently, iCAFs were implicated in mediating resistance to neoadjuvant therapy in rectal cancer.[1] Specifically, upon irradiation, *IL1a* results in iCAF senescence, which in turn leads to ECM accumulation and chemoradiotherapy resistance.

Liver Cancer

Growth factors derived from CAFs, such as *IGF*,[71] hepatocyte growth factor (*HGF*),[72] and epidermal growth factor (*EGF*),[73] contribute substantially to tumor progression. For instance, *IGF2* expressed by fibroblasts boosts a cancer stem cell–like phenotype and mediates resistance to EGF-receptor inhibition in cholangiocarcinoma.[71] *HGF* from hepatic myofibroblasts can activate MET proto-oncogene, receptor tyrosine kinase (c-MET) and mitogen-activated extracellular signal-regulated kinase (MEK)-extracellular regulated MAP kinase (ERK)1/2 pathways in HCC cells, which is associated with poor prognosis of HCC.[72] Human liver myofibroblasts also produce heparin-binding *EGF* and promote the progression of intrahepatic cholangiocarcinoma.[73] Moreover, CAF-secreted cardiotrophin like cytokine factor 1 (*CLCF1*) induces expression of *CXCL6* and *TGFβ* in tumor cells, which further stimulates tumor cell stemness and neutrophil infiltration. In addition, the positive feedback loop built by CAFs, tumor-associated neutrophils, and tumor cells greatly worsens patient outcomes.[7]

A single-cell study conducted in human intrahepatic cholangiocarcinoma (ICC) uncovered 6 subpopulations of CAFs, of which the majority were *CD146*-positive vascular CAFs. These CAFs express high levels of *IL6*, which elicit significant epigenetic alterations in ICC cells. Moreover, ICC cell–derived exosomal *miR-9-5p* induces expression of *IL6* in vascular CAFs to promote tumor progression.[47] Another study showed that myCAFs promote tumor growth via hyaluronan synthase 2. In addition, iCAF-expressed *HGF* enhances ICC growth via tumor-expressed *HGF* receptor MET proto-oncogene, receptor tyrosine kinase (*MET*).[74]

Similar to PDAC,[44] liver metastasis generated by injection of PDAC and CRC cell lines into the hemispleen also showed the existence of iCAFs and myCAFs. Furthermore, genetically engineered mouse models showed that iCAF-secreted *HGF* and myCAF-secreted hyaluronan exert a tumor-promoting role. By contrast, myCAF-expressed type I collagen could suppress tumor growth by mechanically limiting tumor spread, providing a mechanistic explanation for the dual functions of CAFs in cancer.[75]

Esophageal Squamous Cell Carcinoma

CAFs play vital roles in esophageal squamous cell carcinoma (ESCC) progression. For example, Wnt family member (WNT) signaling plays a crucial role in CAF-induced effects. *WNT2* secreted by CAFs promotes tumor progression in ESCC by activation of the Wnt/β-catenin signaling pathway.[76] Accumulating evidence also has indicated that CAFs are closely related to therapeutic resistance through active biochemical signaling or mechanical obstruction of drug delivery.[38] CAFs could confer radioresistance by regulating the DNA damage response.[38] In addition, CAFs could interact with immune cells and impact immunologic environments in ESCC. For example, CAFs could impact $CD8^+$ T-cell infiltration through ECM remodeling. Our group previously reported that *TGFβ1* produced by CAFs increases the expression of laminin γ2 (one of the ECM components) of ESCC cells, which further blocks T-cell infiltration into tumor tissue.[42] Moreover, dendritic cells play an essential role in the efficient antitumor immune responses,[77] and CAF-secreted *WNT2* suppresses the dendritic cell–mediated antitumor T-cell response via the suppressor of cytokine signaling 3 (SOCS3)/phosphorylated Janus kinase 2 (p-JAK2)/p- phosphorylated signal transducer and activator of transcription 3 (STAT3) signaling cascades.[78]

Single-cell transcriptomic analysis of primary ESCC tumor and lymph node metastasis identified 4 subtypes of CAFs. apCAFs are marked by *CD74, HLA-DRA*, and *HLA-DPB1*, and iCAFs have high expression of *IL6*, hyaluronan synthase 1 (*HAS1*), and *COL14A1*. Of note, the other 2 subclusters are myCAFs with remarkable levels of thrombospondin 2 (*THBS2*), *COL12A1*, and Thy-1 cell surface antigen (*THY1*). One of the myCAFs express immunoglobulin genes (*IGKC*), another expresses ECM components and matrix metallopeptidase genes including *POSTN*, *COL1A1*, *CTHRC1*, *MMP1*, and *MMP11*, implying their role in adhesion and migration.[48]

Gastric Cancer

Secretory factors from CAFs may be potential therapeutic targets of cancer. For example, *IL6* induces chemoresistance by activating the *Jak1–STAT3* signaling pathway in GC cells, which provides a rationale for the application of *IL6* inhibitors to enhance the responsiveness to chemotherapy in GC.[79] Moreover, loss of Trimethylation of histone H3 at lysine 27 (h3K27me3) in CAFs results in the release of multiple stem-cell niche factors such as *WNT5A* and *IGF2*, promoting cancer cell growth and migration.[80] In addition, exosomal *miR-522* generated by CAFs inhibits ferroptosis and impairs the chemosensitivity to cisplatin of GC.[81]

In gastric cancer, 3 CAF clusters (fibroblast subcluster*1–3*) have been shown, and all of them increase in a stage-dependent manner. Two clusters show up-regulation of *FAP*, *ACTA2*, and *TAGLN* while another cluster shows

up-regulation of only chondroitin sulfate proteoglycan 4 (*CSPG4*). Further correlation analysis showed that the INHBA–FAP axis is a CAFs regulator in gastric cancer,[10] which is consistent with previous findings regarding CAF-mediated *INHBA* signaling in gastric cancer.[82]

Conclusions and Perspectives

CAFs account for a large proportion of cells in gastrointestinal tumors. Functionally, CAFs play multifaceted roles in tumor initiation and progression. At the same time, CAFs seriously affect the treatment response and prognosis of patients. Therefore, the study of CAFs will contribute to an in-depth understanding of the occurrence, development, and clinical treatment of cancer. In view of the high heterogeneity of CAFs, we encourage future research to be more refined to study the origin and corresponding function of a certain CAF population, which will help to improve our knowledge of CAFs and provide a theoretical basis for future clinical applications.

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